MOLECULAR ANALYSIS OF ENTEROLYSIN A AND *ENTL* GENE CLUSTER FROM NATURAL ISOLATE *ENTEROCOCCUS FAECALIS* BGPT1-10P

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Veljović K., A. Terzić-Vidojević, M. Tolinački, M. Kojić and Lj. Topisirović (2013): *Molecular analysis of enterolysin a and entl gene cluster from natural isolate Enterococcus faecalis bgpt1-10p.* Genetika, Vol 45, No. 2, 479-492.

Strain *Enterococcus faecalis* BGPT1-10P was isolated from artisanal semi-hard homemade cheese from Stara Planina, Serbia. Results showed that BGPT1-10P synthesized a heat labile bacteriocin with a broad spectrum of activity, including *Listeria* and *Candida* species. Further analysis revealed that synthesized bacteriocin is enterolysin A. Moreover, the *entL* gene encoding enterolysin A was found to be located on the chromosome. The *entL* gene was cloned and sequenced. Analysis of nucleotide sequence showed that the *entL* gene in natural isolate *En. faecalis* BGPT1-10P is identical to that of the *entL* gene described previously in *En. faecalis* LMG 2333. Within the cloned DNA fragment containing the *entL* gene, four ORFs were detected. One of them was identified as the *scpE* gene, which encodes a virulent factor staphopain peptidase. Functional analysis of the *entL* gene showed that the complete genetic information necessary for the synthesis of enterolysin A were directly linked solely to it. Strain BGPT1-10P also synthesized gelatinase and citolysin, and contained a set of virulent factors. In addition, BGPT1-10P carries the *ermB* and *tetM* genes conferring the resistance to erythromycin and tetracycline, respectively.

Key words: *Enterococcus faecalis* / enterolysin A / *scpE* gene / antibiotic resistance.

INTRODUCTION

Genus *Enterococcus* is a part of the natural intestinal microflora of humans and animals and is very important in the maintaining the balance of it (LEROY *et al*., 2003). Enterococci have exceptional ability to adapt to inhabit different ecological niches (GIRAFFA, 2003). The data dealing with the basic characteristics and properties of enterococci demonstrate that they possess the so-called "dualistic" effect. It is known that enterococci generally participate in the

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fermentation of food of plant and animal origin and synthesize bacteriocins with excellent antibacterial effect (FRANZ *et al*., 1999). Enterococcal bacteriocins, so-called enterocins, are the subject of detailed study for many years (GÁLVEZ *et al.,* 2007). Their diversity, ecological and practical importance in biopreservation of foods is the objects of investigation in recent years (FRANZ *et al.,* 2007).

On the other hand, enterococci are regarded as indicators of undesirable contamination as well as the microorganisms, which can carry the pathogenic potentials (GIMNÉZ-PEREIRA, 2005). Enterococci are often involved in food spoilage and the spread of antibiotic resistance via the food chain (GIRAFFA, 2002). Detection of virulence factors and antibiotic resistance genes with immune-compromising patients leads to the fact that enterococci may be the cause of many diseases (FRANZ *et al.,* 2003). Fortunately, these characteristics are strain specific and are mostly found among clinical isolates of enterococci. However, studies performed in the recent decades indicated the presence of virulence factors in enterococci isolated from food.

In addition, the presence of the same virulence factor in clinical isolates of enterococci and in the strains isolated from food was confirmed by the PCR method (EATON and GASSON, 2001). Moreover, the research over last decade showed that enterococci present in the food could be reservoirs of antibiotic resistance genes (OGIER and SERROR, 2008). One of today's most pressing problems is the risk of vancomycin-resistant enterococci (VRE). Resistance to chloramphenicol, tetracycline and erythromycin was found in *En. faecalis*, isolated from dairy products. Often detection of antibiotic resistance in enterococci from dairy products is a result of mutations or the acquisition of new gene(s) as consequence of an efficient transfer of genes encoding the resistance among other bacteria and enterococci due to transposons and plasmids carrying those genes (TEUBER *et al*., 1999; HOLLENBECK and RICE, 2012).

The aim of this work was to characterise an enterocin-producing natural *En. faecalis* BGPT1-10P isolated from homemade cheese. The localization of the gene encoding enterolysin A and an analysis of the cloned DNA fragment carrying it was carried out. In addition, the presence of the genes encoding antibiotic resistance as well as virulence factors was also examined.

MATERIALS AND METHODS
Bacterial strains, media and growth conditions

The bacterial strains and plasmids used in this study are listed in Table 1. *Enterococcus faecalis* BGPT1-10P was isolated from semi-hard homemade cheese using standard microbiological procedures. Preliminary strain determination was done according to physiological tests, and ability of strain to perform the hydrolysis of esculin and form black areas on bile-esculin agar. Further taxonomic determination of BGPT1-10P was done by species-specific PCR for enterococci species (VALENZUELA *et al.,* 2009). Enterococcal strains were grown at 30°C in M17 medium (Merck GmbH, Darmstadt, Germany) supplemented with 0.5% glucose (GM17). *Escherichia coli* DH5α (HANAHAN, 1983) was grown in Luria broth (LB) medium at 37°C (SAMBROOK *et al*., 1989). To each medium, agar (1.5% w/v; Torlak, Belgrade, Serbia) was added when used as a solid medium.

Detection and characterization of bacteriocin produced by *En. faecalis* BGPT1-10P

A bacteriocin-producing ability of *En. faecalis* BGPT1-10P was tested using an agar well diffusion assay (HARRIS *et al.,* 1989), with *En. faecalis* BG221 as an indicator strain. Plates

were incubated for 24 h at 30°C. A clear zone around the well, but not near the protease crystal, was taken as a positive signal for bacteriocin production. A preliminary characterization of the bacteriocin was performed, using an overnight culture supernatant, which was tested for stability to heat and pH. For heat sensitivity, 1 ml samples were heated from 40°C to 100°C with increase of 10°C for 15 min each. To test pH sensitivity, 1 ml aliquots of active supernatants were adjusted to different pH values (from 2 to 14) with 1 M NaOH or 1 M HCl. The activity of treated supernatant was explored by agar well diffusion assay.

Table 1. Bacterial strains and plasmids used in this study

Bacterial strains, plasmids	Relevant characteristic(s)	Source or reference
En. faecalis BGPT1-10P	Natural isolate from soft homemade cheese, Bac+	VELJOVIC *et al.,* 2009
En. faecalis BGZLS10-27	Natural isolate from soft homemade cheese, Bac⁻	VELJOVIC *et al.,* 2009
En. faecalis BG221	Natural isolate from soft homemade cheese	VELJOVIC *et al.,* 2009
E. coli DH5α	F⁻, Δ*lac, U169(Φ80 lacZ ΔM15), supE44, hsdR17, recA1, gyrA96, endA1, thi–1, relA1*	HANAHAN, 1983
pGEM-T Easy	Amp^r, *lacZ,*	Promega, Madison, USA
pA13	Em^r, *lacZ*, derivative of plasmid pA1, 4601 bp	KOJIC *et al*., 2010

Bac+ − production of bacteriocin   Bac⁻ − no bacteriocin prodaction

Molecular techniques

Molecular cloning techniques like ligation, PCR amplification and agarose gel DNA electrophoresis were carried out essentially as described previously (SAMBROOK *et al*., 1989). Digestion with restriction enzymes was conducted according to the supplier's instructions (Fermentas, Vilnius, Lithuania). Plasmids from *E. coli* were isolated using the QIAprep Spin Miniprep kit according to the manufacturer's recommendations (QIAGEN, Hilden, Germany). Plasmid DNA from enterococci was isolated using method for isolation of large plasmid described by O'SULLIVAN and KLAENHAMMER (1993). The DNA fragments from agarose gels were purified using QIAqick Gel extraction kit as described by the manufacturer (QIAGEN). *E. coli* DH5α competent cells were prepared using chemical treatment and transformed by heat shock transformation. Selection of transformants was performed by using antibiotic ampicillin (100μg/ml for *E. coli*) and erythromycin (5 μg/ml for enterococci or 250 μg/ml of for *E. coli*). *En. faecalis* BGZLS10-27 electrocompetent cells were prepared and transformed as previously described (SHEPARD and GILMORE, 1995) using Eppendorf Electroporator (Eppendorf, Hamburg, Germany).

DNA sequencing and analysis

For the amplification of the *entL* gene encoding enterolysin A in *En. faecalis* BGPT1-10P, a pair of primers EntlA3 and EntlA9 was used (Table 2). PCR conditions included a hot start of 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 15 s, annealing at 55°C for 30 s and polymerization at 72°C for 1 min. The presence of genes involved in the resistance to erythromycin and tetracycline was determined by using PCR with appropriate

primers (Table 2). PCR amplicons were separated by electrophoresis on a 1% agarose gels and visualized by ethidium bromide staining under UV illuminator.

Table 2. Specific primers used for PCR amplification

Primer	Sequence	Annealing temperature t°C	Source or reference
Primers for *enlA* gene			
EntlA3	5'-GGACAACAATTCGGGAACACT-3'	55°C	NIGUTOVA *et al*., 2007.
EntlA9	5'-GCCAAGTAAAGGTAGAATAAA-3'		
Primers for inverse PCR			
Ent1inv	5'-CAGTGTTCCCGAATTGTTGTCC-3'	50°C	This study
Ent2inv	5'-TTTATTCTACCTTTACTTGGC-3'		
Primers for *orf 2*			
P1FW	5'-GCTAAATATGGTCATGCG-3'	50°C	This study
P2RV	5'-CATCTAATGCTTCATGAG-3'		
Primers for *entL* gene			
P3FW	5'-CTGGTTGTTATTAATCTA-3'	50°C	This study
P4RV	5'-TTATACTCGTTCACCTCT-3'		
Primers for *orf 3*			
P5FW	5'-AGAAAATGACAGTAGGAT-3'	50°C	This study
P6RV	5'-GCAGAGCCTATAAGTATT-3'		
Primers for *orf* 4			
P7FW	5'-AACCGATTGGATGGAACC-3'	50°C	This study
P8RV	5'-ACTTCTGACATGCATGACT-3'		
Primers for *tetK* gene			
TETK-FW1	5'-TTATGGTGGTTGTAGCTAGAAA-3'	55°C	GEVERS *et al.,* 2003
TETK-RV1	5'-AAAGGGTTAGAAACTCTTGAAA-3'		
Primers for *tetL* gene			
TETL-FW3	5'-GTMGTTGCGCGCTATATTCC-3'*	55°C	GEVERS *et al.,* 2003
TETL-RV3	5'-GTGAAMGRWAGCCCACCTAA-3'*		
Primers for RPP genes except *otrA*			
RIBO2-FW	5'-GGMCAYRTGGATTTYWTIGC-3'*	„Touchdown"	AMINOV *et al*., 2001
RIBO2-RV	5'-TCIGMIGGIGTRCTIRCIGGRC-3'*		
Primers for *ermA*			
ERMA-FW	5'-AAGCGGTAAACCCCTCTGA-3'	55°C	STROMMENGER *et al.,* 2003
ERMA-RV	5'-TTCCCAAATCCCTTCTCAAC-3'		
Primers for *ermB*			
ERMB-FW	5'-CATTTAACGACGAAACTGGC-3'	55°C	JENSEN *et al.,* 1999
ERMB-RV	5'-GGAACATCTGTGGTATGGCG-3'		
Primers for *ermC*			
ERMC-FW	5'-AATCGTCAATTCCTGCATGT-3'	55°C	STROMMENGER *et al*., 2003
ERMC-RV	5'-TAATCGTGGAATACGGGTTTG-3'		

*R = A or G; W = A or T; Y = C or T; M = A or C; I = inozin

Southern blot analyses

Total DNA from *En. faecalis* BGPT1-10P was isolated by the procedure described by HOPEWOOD *et al.* 1985. with slight modifications. Isolated DNA was digested by *Hin*dIII restriction endonuclease, separated by gel electrophoresis in 1% agarose and blotted to nylon membranes as described by MANIATIS *et al.* (1982). Prehybridization and hybridization was done as described by the BOEHRINGER MANNHEIM manual for the DIG DNA labelling and detection kit (Roche Diagnostics GmbH, Mannheim, Germany). Membranes were probed with PCR-amplified *entL* structural gene labelled by incorporation of digoxigenin-11-dUTP (Roche Diagnostics GmbH) following the manufacturer's instructions. Hybridization was carried out at 65°C.

Determination of the *entL* gene cluster flanking sequences

To determine the sequences upstream and downstream of the *entL* gene, inverse PCR was carried out with Ent1inv and Ent2inv primers (Table 2) using as template DNA of *En. faecalis* BGPT1-10P digested with *Hin*dIII restriction enzyme, precipitated with ethanol and resuspended in water. Obtained DNA fragments were ligated overnight at 16°C in 20 μl reaction mixture. After ligation, the mixture is precipitated with ethanol and then used as template for PCR. The resulting purified PCR products were passed through a QIAquick PCR Purification KIT/250 (QIAGEN GmbH, Hilden, Germany), then cloned in pGEM-T Easy vector (Promega, Madison, USA). The fragment of 3.5 kb was sequenced in Macrogen's sequencing service (Seoul, Korea). Based on the obtained sequences, the corresponding primers were synthesized (Table 2) and used to recheck unknown surrounding sequences by inverse PCR.

The BLAST algorithm was used to determine the most related sequence relatives in the NCBI nucleotide sequence database (http://www.ncbi.nlm.nih.gov/BLAST). The DNA Strider program was used for open reading frame (ORF) prediction.

RESULTS

Characterization of *Enterococcus faecalis* BGPT1-10P

Strain *Enterococcus faecalis* BGPT1-10P isolated from semi-hard homemade cheese exhibited a broad spectrum of activity towards Gram-positive bacteria. BGPT1-10P inhibited among others the growth of *Listeria monocytogenes*, *L. innocua* and *Candida pseudotropicalis* (VELJOVIC *et al.*, 2009). Bacteriocin of the strain BGPT1-10P was heat labile losing an activity after incubation for 15 min at 60°C and above tested temperature, but not after incubation for the same time at 40°C or 50°C. Testing bacteriocin activities at different pH values showed that it retains activity in the pH range from four to 10.

Since it is known that enterococcal strains produce various bacteriocins in order to identify which bacteriocin(s) produces *E. faecalis* BGPT1-10P, a set of PCR primers was used (Table 2). Results showed that a PCR amplicon was obtained only when enterolysin A-specific primer pair EntlA3 and EntlA9 was used. These two primers amplify nearly entire enterolysin A structural gene giving DNA fragment of 1,007 bp long (Figure 1). The sequence of the PCR-amplified fragment was identical to that described for the enterolysin A gene of *En. faecalis* LMG 2333 (*entA*, GenBank accession number AF249740, NILSEN *et al.* 2003).
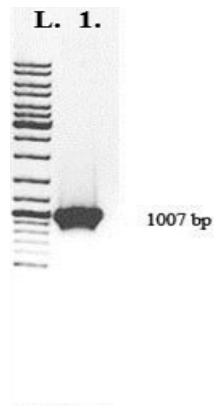
Figure 1. Electrophoresis of DNA fragment after PCR amplification on 1% agarose gel. L. ″Gene Ruler^TM″100 bp DNA Ladder, 2. 1007 bp fragment of *entL* gene after PCR reaction with EntlA3 and EntlA9 primers and chromosomal DNA from BGPT1-10P strain

Localization of structural *entL* genes in strain *Enterococcus faecalis* BGPT1-10P

To make a precise location of the *entL* gene, Southern hybridization was performed using chromosomal and plasmid DNA isolated from *En. faecalis* BGPT1-10P as described in Materials and methods. A hybridization signal was not detected with plasmid DNA. In contrast, a positive hybridization signal was obtained with 4.5 kb long *Hin*dIII fragment of chromosomal DNA, suggesting that the *entL* gene is located on the chromosome of the strain *En. faecalis* BGPT1-10P (Figure 2).

Figure 2. Southern blot hybridization of *entL* gene. L. ″Gene Ruler^TM″100 bp DNA Ladder, 1. PCR amplified *entL* gene probe , 2. Total DNA from BGPT1-10P strain digested with restriction endonuclease *Hin*dIII.

Analysis of the *entL* gene flanking sequences

Using circularized *Hin*dIII fragment of 4.5 kb as a template for the inverse-PCR with primers Ent1inv and Ent2inv, PCR amplification yielded a DNA fragment of 3.5 kb which was overlapped with 1kb long *entL* gene. It was found that the sequence of 4.5 kb contains four open reading frames (ORFs) in addition to *entL* gene. There are two hypothetical proteins (ORF1 and ORF2), a conserved domain protein (ORF3) and part of the *scpE* gene encoding staphopain peptidase. BLAST searching the NCBI database revealed that the partial amino acid sequence of staphopain peptidase from strain BGPT1-10P is the same as deposited for strains of *Staphylococcus aureus* species, as well as of several clinical *En. faecalis* strains (*En. faecalis* TX1302, Acc.No. AEBK01000059.1; *En. faecalis* TX1342, Acc.No. AEBJ01000044.1; *En. faecalis* TX4244, Acc.No. AEBH01000029.1 *itc.*).

The total size of the amplified and analyzed DNA sequence of *En. faecalis* BGPT1-10P was 5,156 bp (Figure 3). The sequence was submitted into the EMBO database, GenBank, Acc.No. HE585879.1.
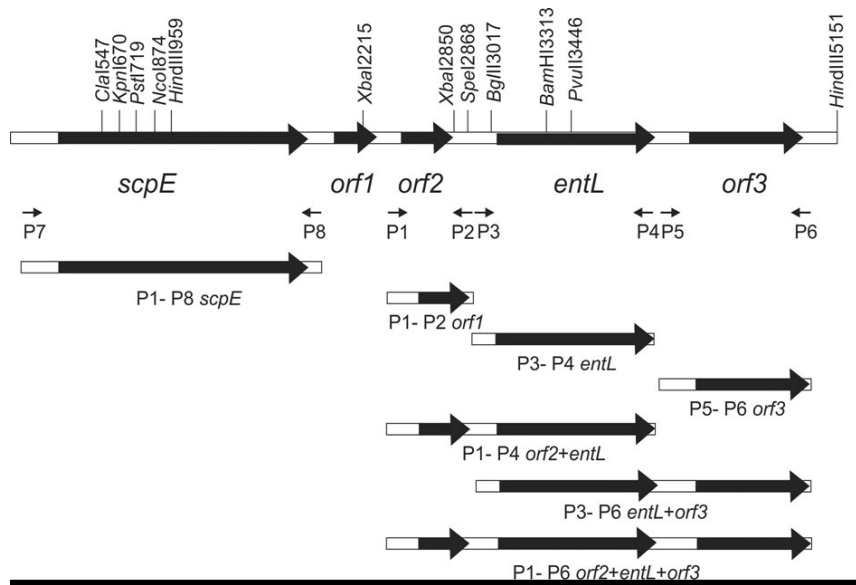

Figure 3. ORFs arrangement and orientation on 5,156 bp DNA sequence, with bacteriocin activity of cloned and expressed fragments in pA13 vector and position of all primers used in PCR reaction for obtaining fragments carrying specific ORFs or their combination.

Note: *scpE* – staphopain peptidase C47 (from 271-1767 bp), *orf1* – hypotetical protein, unknown function (2056-2238 bp), *orf2* – hypothetical protein (2533-2790 bp), *entL* – enterolysin A, peptidase M23 family (3041-4072 bp), *orf3* – conserved domain protein (4243-4773 bp). From P1 to P8: primers position for amplifying *orf*-s or their combination. Bacteriocin activity of all transformants with corresponding constructs are also shown on the map: + - construct with bacteriocin ability; – - construct with no bacteriocin ability.

Functional analysis of the identified ORFs

To perform the functional analysis of the identified ORFs a set of constructs were made by using PCR and appropriate primers (Table 2). This analysis was also used to see whether the *entL* gene solely could encode a synthesis of enterolysin A. Therefore, the genes *orf2*, *entL*, *orf3* and *scpE* were amplified separately or in combinations (*orf2* + *entL*; *entL* + *orf3* and *orf2* + *entL* + *orf3*), and cloned into pGemT-Easy vector. To test the functionality of these genes a re-cloning of *Eco*RI fragments from pGemT-Easy vector into the pA13 vector that has ability to replicate in enterococci was made. Obtained constructs designated as k6 (carrying solely the *entL* gene), k11 (*entL* + *orf3*), k14 (*orf2* + *entL*), k20 (*orf2* + *entL* + *orf3*), k28 (*orf2*), k36 (*orf3*), k20 (*orf2*, *entL* and *orf3*), and k41 (*orf4*) were digested with *Eco*RI to confirm that they are carrying the expected fragments (Figure 4).
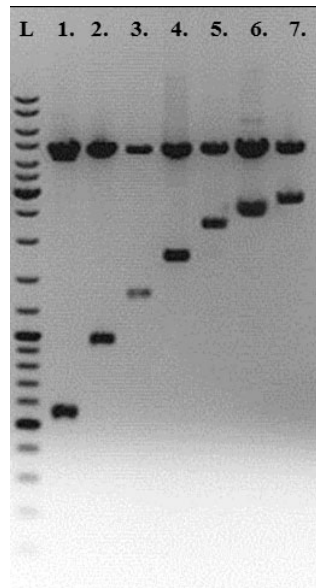

Figure 4.*Eco*RI digestion of all constructs after cloning in pA13 vector; L - ″Gene Ruler^TM″100 bp DNA Ladder, 1. k28 (*orf2*), 2. k36 (*orf3*), 3. k6 (*entL*), 4. k41 (*orf4*), 5. k11 (*entL* and *orf3*), 6. k14 (*orf2* and *entL*), 7. k20 (*orf1*, *entL* and *orf3*).

Transfer of constructs into homologous enterococcal Bac⁻ strain *En. faecalis* BGZLS10-27 revealed that the *entL* gene regardless of the surrounding ORFs is sufficient for the enterolysin A production. It was found that *En. faecalis* BGZLS10-27 harbouring only construct k6 has shown antimicrobial activity (Figure 5).
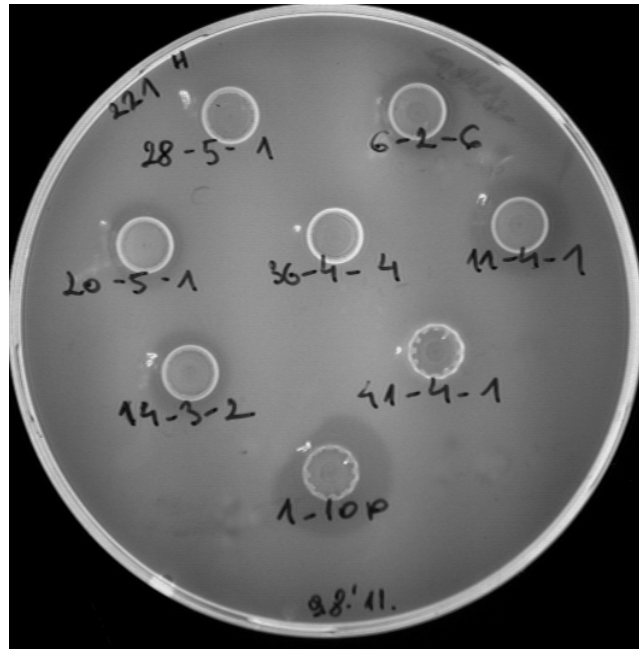
Figure 5. Antimicrobial activity of supernatants from transformants: 28-5-1, 6-2-6, 20-5-1, 36-4-4, 11-1-4,

14-3-2, 41-4-1 and BGPT1-10P as control strain, with BG221 as the indicator strain.

Analysis of nucleotide sequences

Comparative analysis of 5156 nucleotide DNA sequence and all analysed ORF-s showed different homology with the corresponding sequences deposited in NCBI database. The protein encoded by *scpE* gene shows 100% identity with amino acid sequence of staphopain peptidase C47 of *S. aureus* species as well as with clinical isolates of *En. faecalis* (GenBank AEBK01000059.1; AEBJ01000044.1 *et al*.). *Orf1* gene shows 89% homology with gene localized on the plasmid pLG2 of the *En. faecalis* strain, which encode transposase (pLG2-0010) or hypothetical protein (pLG2-0011), resolvase (pLG2-0012) and others. Amino acid sequence encoded by *orf2* gene shows 75% and 54% identity with hypothetical protein from *En. faecalis* JH1 and from *Lb. casei* BL23, respectively. The *entL* gene from *En. faecalis* BGPT1-10P strain and its protein sequence showed 100% identity with enterolysin A, or peptidase M23 family. At the amino acid level, *orf3* gene encoded protein shows 100% identity with conserved hypothetical protein found in clinical isolates of *En. faecalis* JH1, D6 or peptidase C39 family.

Detection of antibiotic resistance genes

Strain BGPT1-10P is resistant to erythromycin (MIC≥4mg/L) and tetracycline (MIC≥ 16 mg/L). PCR method was used to see whether the phenotypic resistance to erythromycin and tetracycline is associated with the presence of known genes. PCR analysis revealed the presence of *ermB* gene product of 425 bp. Obtained PCR fragments were sequenced and computer-assisted alignment of nucleotide sequences with BLAST program showed 97% identity to the *ermB* gene encoding protein ErmB conferring a resistance to erythromycin. In addition, PCR analysis revealed the presence of *tetM* gene that encode tetracycline resistance via a ribosome protection protein mechanism.

DISCUSSION

In this paper, we analysed the strain *En. faecalis* BGPT1-10P isolated from home-made cheese, which is made without the addition of starter cultures in milk. It is shown that this strain synthesized bacteriocin with a broad spectrum of activity, including *Listeria* and *Candida* species and some enterococci and lactococci species. Strain BGPT1-10P does not show activity against tested lactobacilli as well as the pathogenic species of *E. coli* and *S. aureus* (VELJOVIC *et al.,* 2009).

It has been shown that strain BGPT1-10P synthesize a heat labile enterocin and also contains the gene encoding enterolysin A (*entL*). The *entL* gene in BGPT1-10P is located on the chromosome, on the 4.5 kb *Hin*dIII fragment. It has been shown previously that genes encoding some bacteriocins like helveticin J (JOERGER and KLAENHAMMER, 1990) and enterocin AS-48RJ (ABRIOUEL *et al.,* 2005) are localized on chromosome. The analysis of the *entL* gene flanking sequences of natural isolates BGPT1-10P, shown a unique sequence that contained four potential genes (*orf1*, *orf2*, *entL* and *orf3*), and the gene for staphopain peptidase (*orf4*). Similar order sequences of the genes were found in clinical enterococcal genomes, whose sequences are deposited in the database. As regards the homology of genes, it was shown that is a hypothetical protein ORF2 of the strain BGPT1-10P, differs from the same protein from *En. faecalis* clinical strains JH1, D6 and others. The percentage of identity ORF2 was 75% with hypothetical protein *En. faecalis* strains JH1 or 54% identity with hypothetical protein originating from the strain *Lb. casei* BL23. At the same time, and the nucleotide sequence of *orf1* gene shows 89% homology with genes localized on plasmid pLG2 strain originating from *En. faecalis*.

Orf4 gene encodes a protein, which is 100% identical to staphopain peptidase C47, which was initially characterized for the species *S. aureus* (ARVIDSON *et al.,* 1973). According to CHEUNG and colleagues (1997) suggested that this enzyme is designated as a virulent factor, and it's present in several clinical isolates of *En. faecalis* species deposited in the database. In this paper, *orf4* gene was identified as *scpE* staphopain peptidase gene of natural isolates BGPT1-10P. This data suggests a strong similarity of enterococci and staphylococci species, and their common origin. At the same time, points to the fact that enterococci have a strong ability to communicate with other bacteria, and efficient transfer of genetic elements, and thus explains the fact that they possess genetic elements specific to pathogenic species. Functional analysis showed that solely the *entL* gene encodes the complete genetic information, necessary for the synthesis and activity of enterolysin A.

In addition, fact that all present genes in strain BGPT1-10P are found in other enterocci, but in different order indicate possibility that genes are not transferred in one step as a cluster. It seems that certain organization of genes in the strain BGPT1-10P occurred as consequence of

several independent events of gene transfer or gene transfer combined with subsequent genetic rearrangements.

Based on data from the literature it is known that different groups of independent scientists characterized the same bacteriocin (SÁNCHEZ *et al.*, 2007; DE KWAADSTENIET *et al.,* 2006). These results showed that the same bacteriocin is found in different species, which are not only distant in terms of classification, but also inhabit very different habitats. Strain BGPT1-10P seems to be identical to *En. faecalis* strain DPC5280, which was isolated from Irish raw milk (HICKEY *et al.,* 2003). Both strains synthesized enterolysin A, citolysin without beta-hemolytic activity, gelatinase and both strains showed good antimicrobial activity, acting on a number of different bacteria. In addition, both strains are rich in a variety of virulence determinants and resistant to many antibiotics (HICKEY *et al.,* 2003; VALENZUELA *et al.,* 2009). It is obvious that the effect of gene transfer was a very large, which allows for strains that are temporally and spatially distant become similar, and at the same time may explain why some strains are rich in variety of virulence factors and resistant to many antibiotics. Since the strain BGPT1-10P are natural isolate, successful gene transfer may explain why this strain is full of virulent factors. In addition, BGPT1-10P is resistant to tetracycline and erythromycin (VALENZUELA *et al.,* 2009). These antibiotics have wide application in humans and in animals. In this work, we determined the molecular basis of this resistance. The BGPT1-10P carries the *ermB* gene responsible for resistance to erythromycin and *tetM* gene responsible for tetracycline resistance.

Thanks to the great diversity of enterococci strains, and their possessing of different functional characteristics, it is necessary to analyse each individual strain, prior to its use in food or medicine.

ACKNOWLEDGEMENT

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MOLEKULARNA ANALIZA ENTEROLIZINA A I *ENTL* GENSKOG KLASTERA PRIRODNOG IZOLATA *Enterococcus faecalis* BGPT1-10P

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Izvod

Soj *Enterococcus faecalis* BGPT1-10P je izolovan iz domaćeg polutvrdog sira, poreklom sa Stare Planine. Rezultati pokazuju da soj BGPT1-10P sintetiše termolabilan bakteriocin, enterolizin A, sa širokim spektrom delovanja, uključujući patogene bakterije roda *Listeria* i *Candida*. *EntL* gen, odgovoran za sintezu ovog bakteriocina, je hromozomalno lokalizovan. Analiza nukleotidne sekvence *entL* gena kod prirodnog izolata *En. faecalis* BGPT1-10P je identična sa *entL* genom soja *En. faecalis* LMG 2333, koji je prethodno okarakterisan. Pokazana je jedinstvena sekvenca *entL* gena i njegove okoline, koju čine *orf1*, *orf2* i *orf3* geni, kao i *scpE* gen. Prvi put je kod prirodnog izolata okarakterisan *scpE* gen, koji kodira virulentni faktor stafopain peptidazu. Funkcionalna analiza *entL* gena je pokazala da je kompletna genetička informacija, neophodna za sintezu i aktivnost enterolizina A, sadržana u *entL* genu. Soj BGPT1-10P osim enterolizina, sintetiše i želatinazu i citolizin i sadrži set različitih virulentnih faktora. Pored toga, BGPT1-10P nosi *ermB* i *tetM* gene, odgovorne za rezistenciju na eritromicin i tetraciklin.